The Effects of Hyperbaric Oxygen Therapy Against Streptomycin Ototoxicity

Streptomisin Ototoksisitesine Karşı Hiperbarik Oksijen Tedavisinin Etkileri

Oxygen Therapy / Oksijen Tedavisi

Salih Bakır[1], Musa Özbay[1], Vefa Kınış[1], Ediz Yorgancılar[1], Ramazan Gün[1], Aylin Gül[1], Ulaş Alabalık[2], İsmail Topçu[1]
[1]Kulak Burun Boğaz Hastalıkları Anabilimdalı, [2]Patoloji Anabilimdalı, Dicle Üniversitesi Tıp Fakültesi, Diyarbakır, Türkiye

Özet

Amaç: Hiperbarik oksijen (HBO), önemli antioksidan aktivitesi nedeniyle çeşitli hastalıkların tedavisinde giderek artan oranda kullanılmakta olan bir adjuvan tedavi metodudur. Bu deneysel çalışmada streptomisine bağlı ototoksisitenin önlenmesinde HBO tedavisinin etkinliğinin araştırılması amaçlanmıştır. Gereç ve Yöntem: Yirmi sekiz adet erişkin Wistar albino rat randomize olarak 4 gruba ayrıldı: Kontrol (n=7), streptomisin (n=7), HBO (n=7) ve streptomisin+HBO (n=7). HBO uygulanan sıçanlar basınçlı tank içine yerleştirildi ve yedi günlük bir süre içinde günde 60 dakika boyunca 2.5 mutlak atmosfer basınçta % 100 oksijen almaları sağlandı. Sıçanların işitme düzeyleri HBO uygulaması başlamadan önce ve son gün distortion product otoakustik emisyon (DPOAE) ile test edildi. 7. günde son DPOAE ölçümleri takiben genel anestezi altında tüm gruplardaki hayvanlar sakrifiye edildi. DPOAE amplitüdlerindeki farklılıklar belirlenerek işitme sonuçları istatistiksel olarak analiz edildi. Ayrıca, her sıçanın kokleası hematoksilen ve eozin (H&E) ile histopatolojik olarak ışık mikroskobu altında değerlendirildi. Bulgular: Kontrol ve HBO grubunda histopatolojik değerlendirmede koklear tüylü hücrelerin çoğunlukla korunduğu gözlendi. DPOAE sonuçlarında ise tedavi öncesi ve sonrası arasında anlamlı bir fark saptanmadı (p>0.05). Öte yandan, streptomisin ve streptomisin+HBO uygulanan sıçanlarda hem tüylü hücrelerde hemde işitme fonksiyonlarında kayıp saptandı (p<0.05). Streptomisin+HBO grubunun DPgram sonuçları, streptomisin grubunun DPgram sonuçları ile karşılaştırıldığında anlamlılık yoktu (p>0.05). Tartışma: Antioksidan özellikli HBO'nun koklear tüylü hücreler üzerinde zararlı bir etkisi gözlenmemiştir. Öte yandan, sıçanlarda oluşturulan streptomisine bağlı koklear hasara karşı da koruyucu bir etkisi olmadığı sonucuna varılmıştır.

Anahtar Kelimeler

Aminoglikozid; Streptomisin; Ototoksisite; İşitme Kaybı; Hiperbarik Oksijen

Abstract

Aim: Hyperbaric oxygen (HBO) is an important adjuvant therapy and being increasingly used in the treatment of various disorders because of having an important antioxidant activity. This experimental study was designed to determine the possible protective effect of HBO therapy on streptomycin-induced ototoxicity. Material and Method: Twenty-eight adult Wistar albino rats were divided into four groups: Streptomycin (n=7), saline (n=7), HBO (n=7), and streptomycin plus HBO (n=7). The HBO administered rats were placed into a large pressure chamber and received 100% oxygene at 2.5 atmosphere absolute for 60 minutes per day in a period of seven days. Rats were tested with DPOAE (Distortion Product Otoacoustic Emissions) in the beginning and the end of study. The animals in all groups were sacrificed under general anesthesia on the seventh day. Biopsy specimens from inner ear were stored for histopathologic examination with hematoxylin and eosin (H&E) under light microscopy. Results: Outer hair cells shown by light microscopic images were mostly preserved in control and HBO group. DPOAE measurements revealed no significant differences between the beginning and the end (p>0.05). Streptomycin and streptomycin plus HBO treated rats showed loss of hair cells and auditory functions significantly (p<0.05). Between the groups of streptomycin and streptomycin plus HBO; there was no statistically significance according to the analysis of the histopathological scores and DPgram results (p>0.05). Discussion: HBO has probably no harmful effect on hair cells. But it seems to be not beneficial in a streptomycin-induced cochlear damage rat model.

Keywords

Aminoglycoside; Streptomycin; Ototoxicity; Hearing Loss; Hyperbaric Oxygen

DOI: 10.4328/JCAM.1250 Received: 11.08.2012 Accepted: 26.08.2012 Printed: 01.03.2014 J Clin Anal Med 2014;5(2): 97-102
Corresponding Author: Salih Bakır, Department of Otolaryngology, Dicle University Medical College and MD, Department of Physiology, Dicle University Medical College 21280, Diyarbakir, Turkey. T.: +90 4122488001/4492 F.: +90 4122488440 E-Mail: drsalihbakir@gmail.com

Introduction

After almost eight decades in clinical use, although the ototoxic effects of aminoglycosides in animals and humans are well documented and also high occurrence risk of another serious complication such as nephrotoxicity, this class of drugs seems still likely to remain an important component of medical treatment options due to: 1) the effectiveness at the treatment of gram-negative aerobic bacterial infections, 2) being an alternative medication among the rare options of treatment in tuberculosis, 3) beneficial effects in Meniere's disease and 4) relatively low cost [1-6].

Aminoglycoside antibiotics may injure the outer hair cells at the basal turn of the cochlea and cause sensorineural hearing loss. In more severe cases, the damage may progress to inner hair cells and supporting cells [4-6]. Therefore, it is important to identify the development mechanism and subsequently prevention methods against to vestibulotoxicity and cochleotoxicity. But no therapy currently exists to inhibit or reduce the potential ototoxicity of parenterally aminoglycoside antibiotics, although improved therapeutic regimens have lowered the risk. For this aim, a great effort has been made and several studies have been conducted to develop strategies and to find a way out against to aminoglycoside-induced ototoxicity. Among them, anti-free radical agents are becoming increasingly important [4-5].

Hyperbaric oxygen (HBO) therapy, having an important antioxidant activity, is based on inhalation of 100% oxygen under pressures above 1 atm (pressurized between 1.5 and 3.0 atmosphere absolute) in a pressure chamber [7,8]. Due to its antioxidant property, HBO has been considered to prevent ototoxicity. With the possible otoprotector effect, it could become a valuable therapeutic tool. Although the mechanism of HBO therapy in inner ear has not been still fully understood, today, it is an important adjuvant therapy and being increasingly used in a variety of disorders, such as severe anaemia, decompressive diseases, arterial gas embolism, carbon monoxide poisoning, stimulant of wound healing on refractory wounds, gas gangrene, radiation injury, necrotizing infections, and chronic osteomyelitis [7-9]. It has been also used in the management of acute ischemia-related disorders like acute sensorineural hearing loss such as sudden deafness, and acute noise trauma [8,10-15]. But to the best of our knowledge, no study to date have investigated the possible protective effect of HBO against to ototoxic effect of aminoglycoside. The objective of this work was to assess the auditory and histopathologic effects on rat cochlea exposed to aminoglycoside with/without HBO. In addition to histopathological examination with hematoxylin and eosin (H&E) staining, caspase-3 immunoreactivity was also demonstrated for immunohistochemical examination in present study. Because, caspase-3 is more effective than H&E staining to distinguish apoptotic cells. The caspase-3 immunoreactivity is strongly associated with an increased apoptotic event in the cochlea that indicates a malfunction of the hearing organ [16]. In order to test the integrity of hair cells and monitoring aminoglycoside ototoxicity, distortion product otoacoustic emission (DPOAE) method was used.

Material And Method

All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals issued by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [17]. Study was also approved by the Ethical Committee of our institution under permit 2012/09.

Experimental design

Twenty-eight male adult Wistar albino rats with intact Preyer reflexes and normal eardrums weighing between 195-260 g were used in this study. They were maintained according to the standard guidelines. All animals were housed reasonably in cages under standard environmental conditions (room temperature between 22-24°C and 50% relative humidity within a 12-h light/12-h dark cycle photoperiod). All the animals had free access to water and conventional laboratory diet until before sacrifice. The rats were randomly divided into four groups as follows: Control group (n=7), streptomycin group (n=7), HBO group (n=7) and streptomycin plus HBO group (n=7). Placebo (Saline;2.5 mL/kg, IM) was applied in control group. Streptomycin was administered via intramuscular (IM) way (100 mg/kg/day dose) in streptomycin group and streptomycin plus HBO group for seven days until surgery. The rats of HBO group and streptomycin plus HBO group were placed into a large pressure chamber of 20 liters volume separately and received 100% oxygene at 2.5 atmosphere absolute for 60 minutes per day in a period of seven days [18]. The streptomycin plus HBO group was given an IM dose of 100 mg/kg of streptomycin and immediately followed up with 60 min of HBO at 2.5 atm. Anesthesia was not performed and they breathed spontaneously during HBO treatment. The rats of control group and streptomycin group did not receive HBO.

Anesthesia

Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Ketalar®) 60 mg/kg and 2% xylazine hydrochloride (Rompun®) 10 mg/kg by intramuscular injection (IM) before evaluating hearing and before sacrification.

Hearing assessment

In order to test the integrity of hair cells, all animals were tested with DPOAE under anesthesia in a quiet room (less than 50 dB background noise). Before DPOAE was measured, otoscopy was performed to confirm that the external auditory canal and tympanic membrane were normal. Only rats with normal ear canal and tympanic membrane and with initial otoacoustic exam showing normal responses were included in this study. Otoacoustic emission (OAE) recordings were elicited from the right and left ear of each animal utilizing a standard commercial ILO-96 OAE apparatus cochlear emission analyzer (Otodynamics Ltd., London, UK). DPOAE responses are generated when the cochlea is stimulated simultaneously by two pure-tone frequencies (f1 and f2). The data were processed and evaluated with OAE software (EZ Screen 2 Otodynamics OAE Screening and Data Management Software, Hatfield, UK). Each DPOAE test required ~3 min to perform. DPOAEs were recorded before drug administration at first day and before the animals were sacrificed. Following anesthesia, the primary tones were introduced into the animals' outer ear canal through an insert earphone, us-

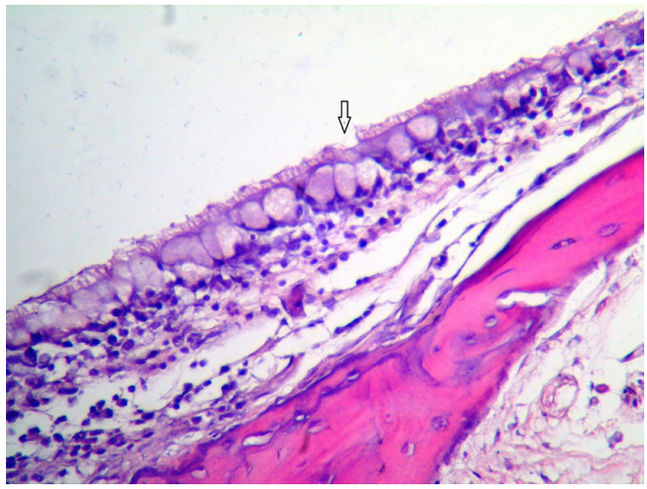

Figure 1. HBO group. Histomorphologic appearance of the hair cells in cochlea. There is no significant differences in architecture compared to the control group (H&E x400) (Arrow: Hair cell losses were demonstrated in this area)

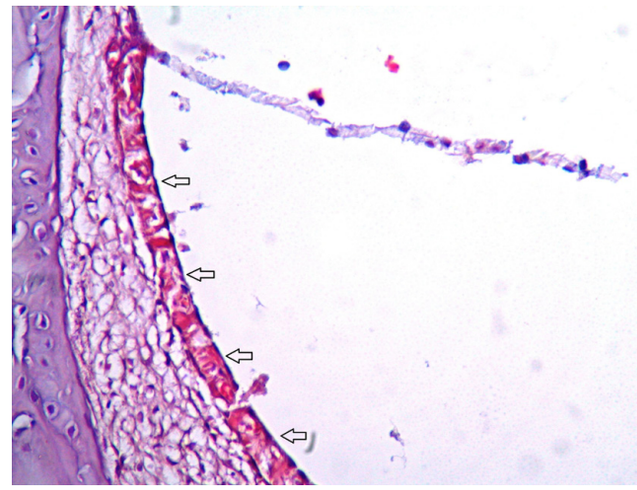

Figure 2. Streptomycin plus HBO group. Severe hair loss was observed in cochlear hair cells. There is no significant differences in architecture compared to the streptomycin group (H&E x400)

ing a plastic adapter that sealed the probe in the outer ear canal. Equilevel primary tones f1 (65 dB) and f2 (55 dB) were fixed at f1/f2 = 1.22, and DPOAEs were measured at five different frequencies ranging from 2000 to 8000 Hz (2002, 3003, 4004, 6006 and 8008 Hz). DPOAEs were determined as DPgrams. By calculating the difference between the distortion products, the signal–noise ratios (SNR) for each frequency were found. The DPOAE response was evaluated using the SNR. Loss or impairment of outer hair cell function results in the attenuation of SNR. For each animal, SNR at five frequencies were recorded. Data were collected separately for each rat and the results were statistically analysed.

Operation procedure

All procedures were performed under clean but nonsterile conditions. After anaesthesized they were decapitated. The skull bone of the subjects were cut in the midline and each temporal bone was dissected by scalpel and a pair of scissors. Using the hands and having the external auditory canal as a guide, the bulla was localized with the thumbs. Their temporal bones containing the tympanic bullas were separated from other structures to reveal the cochleae by holding it with one hand and with a haemostatic clamp. At the end, the cochleas were dissected and removed en-bloc. Biopsy specimens from inner ear were fixed into a 10 % formaldehyde solution and stored for histopathologic and immunohistochemical examinations. Examination was performed by experienced pathologists in blinded fashion.

Tissue Preparation

The cochleas of each rat were fixed for 24 hours in 10% formaldehyde solution and conducted to decalcification for three weeks in 5% formic acid solution. After fixation and decalcification processes, the cochleas were washed with tap water for 24 hours, dehidrated by reaction with graded alcohol series and were transparented and blocked after infiltrated with paraphine. Each paraffin-embedded specimen was sectioned with a microtome (Leica RM2125RTS) at a thickness of 4 µm. Each section was stained with H&E solution and observed with a Nikon ECLIPSE 80i microscope. On the other hand, after keeping in the incubator fixed to 62°C degree for deparaffinization for 60

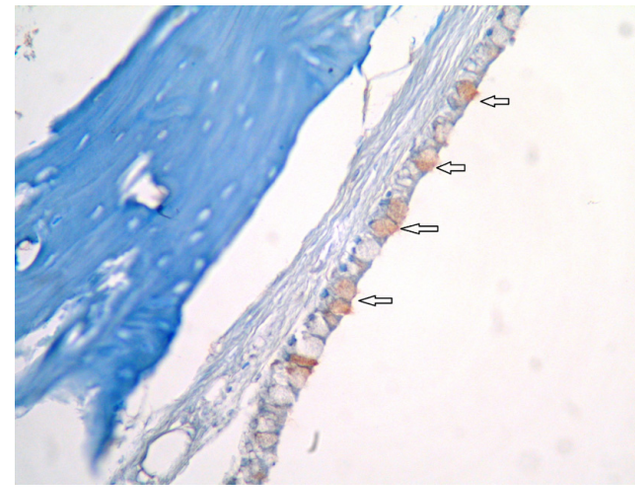

Figure 3. Streptomycin plus HBO group. Caspase-3 positivity (Arrows: Apoptotic cells were shown). (Immunoperoxidase, x400)

minutes, remaining tissue sections were treated with xylene for 4x5 minutes and alcohol solutions with 96% concentration for 4x5 minutes. After deparaffinization process and adding citrate solutions of pH=6, tissue sections were schocked in Biocare's Decloaking Chamber with high pressure being heated to

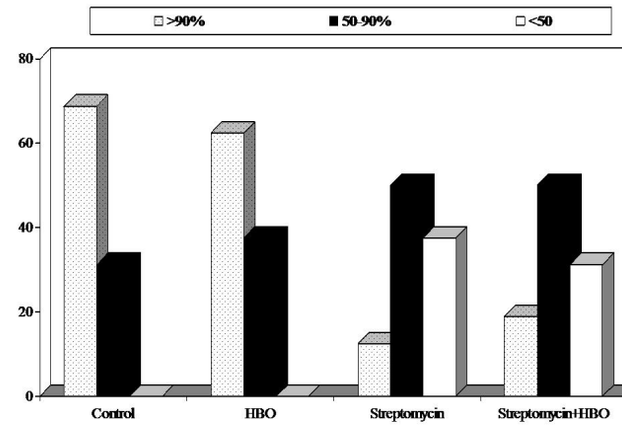

Figure 4. The distribution (percent) of the intact hair cells area in morphological analysis according to the groups. (>90%: mild; more than 90% intact hair cells area of the section, 50-90%: moderate; the rate of the intact hair cells area was between 50%-90%, <50%: severe; less than 50% intact hair cells area of the section.)

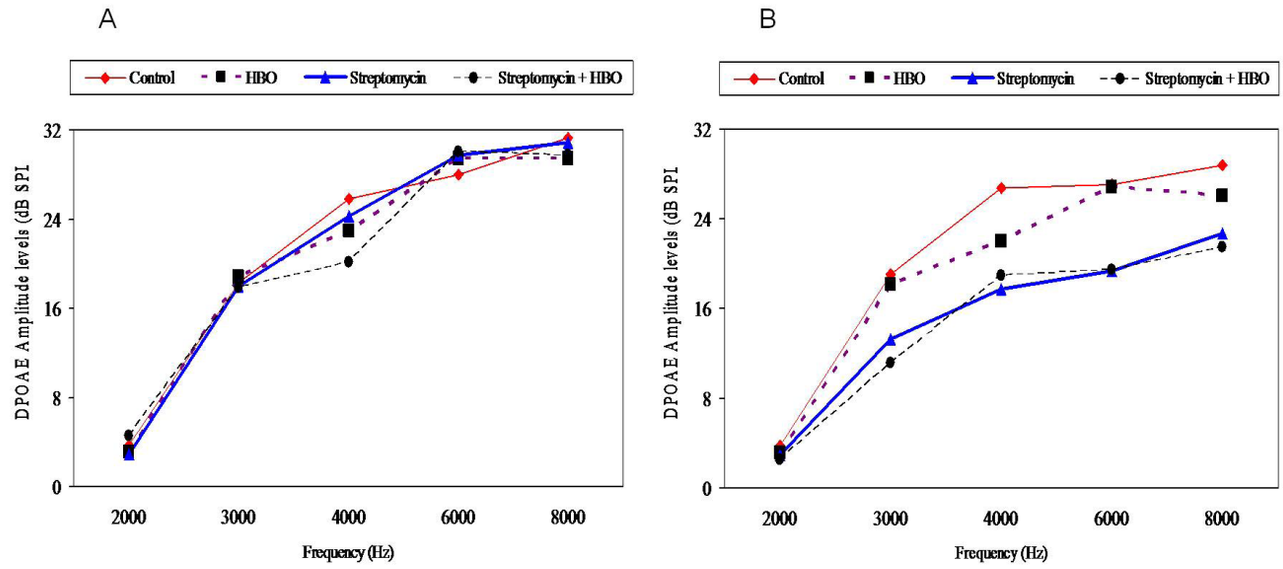

Figure 5. DPgrams from rats treated with saline, streptomycin, HBO and streptomycin plus HBO. A- Determination of DPOAEs on day 0. B- Determination of DPOAEs on day 7. (DP= Distortion Product, DPOAE= Distortion Product Otoacoustic Emission, SPL= Sound Pressure Level)

125°C degree. Then, protein block (Ultra V Block) (ScyTek, USA) was dropped on tissues and those tissues were waited for 20 minutes. Sections were washed with Phosphate Buffered Saline (PBS) solution for 2x5 minutes. Next, the prepared tissue sections were incubated with the primary antibody caspase-3 (CPP32, Thermo Scientific) for 60 minutes. After incubation, the washing process with PBS for 2x5 minutes was repeated. Afterwards, Biotinylated link antibody (ScyTek, USA) was dropped on tissue sections and waited for 20 minutes. Again, washed with PBS solution for 2x5 minutes. Then, the sections were treated with Streptavidin/HRP solution (ScyTek, USA) for 20 minutes and followingly washed with PBS solution for 2x5 minutes. The sections treated with DAB chromogen for 10 minutes were washed with distilled water and then, nuclear adverse staining was done with Mayer's Hematoxylin (Bio-Optica,USA) for 1 minute. Then the sections were washed with distillated water until blueness disappeared, and closed after reacted with alcohol and xylene. The sections were evaluated according to the intensity of caspase-3 immunoreaction under light microscope (Nikon ECLIPSE 80i). In morphological analysis; the intact hair cells area of the section up to; 50% was assessed as severe, 50%-90% was assessed as moderate, more than 90% was assessed as mild. Immunohistochemically, the presence of caspase-3 stained cells were assessed as positive immunoreactivity, the absence of caspase-3 stained cells were assessed as negative immunoreactivity.

Statistical analysis

Statistical evaluation was carried out using the Statistical Package for the Social Sciences for Windows (version 15.0, SPSS Inc.,Chicago, IL, USA). Results were analyzed statistically by Kruskal-Wallis to determine differences in amplitudes of DPOAEs and corresponding noise floor differences and thresholds for each frequency. The histopathological variations of streptomycin, HBO and streptomycin plus HBO and without medication (control) were evaluated and compared each other by Mann Whitney U test. Test and results were expressed as mean ± SD. The level of statistical significance was set at $p < 0.05$.

Results

Histopathological results

According to our results under light microscopy; histopathological examination with H&E staining showed that any deterioration was not observed in cochlear hair cells in control group. Additionally, H&E staining showed no distinctive difference between the control group and the HBO group (p=0,710) (fig 1, fig 4). In streptomycin-treated rats (streptomycin group and streptomycin plus HBO group), cochlear hair cells showed significant hair losses compared to control and HBO group (fig 2, fig 4). No significant differences in average histopathological scores were observed between streptomycin and streptomycin plus HBO groups (p=0,864) (fig 4). With immunohistochemical examination, caspase-3 immunoreactivity was not observed in control group and HBO group (not shown), whereas in both streptomycin group and streptomycin plus HBO group, hair losses and (+) caspase-3 immunoreactivity was observed in organ of corti, supporting cells and basilar membrane (fig 3).

Hearing results

Differences in DPOAE amplitudes, and therefore in cochlear activity, between four groups were revealed. In the DPgrams, for all sessions, the emission amplitude levels were greater than the noise floor throughout the testing frequencies. On day 0, the initial baseline DPOAE measurement results presented similar values while comparing the groups prior to drug administration (p>0.05) (fig 5). On the 7th day, according to the results of the second DPOAE measurements, control and HBO group revealed no significant differences (p>0.05). Whereas in streptomycin and streptomycin plus HBO groups, a prominent decrease in cochlear activity involved frequencies between 2002 and 8008 Hz was observed (p<0.05) (fig 5). When compared to control and HBO groups, DPgram of the streptomycin group and streptomycin plus HBO group was significantly deteriorated (cochlear ac-

tivity was reduced) in the second DPOAE measurement (day 7) ($p<0.05$). Between the groups of streptomycin and streptomycin plus HBO; there was no statistically significance according to the analysis of the DPgram results on day 7 ($p>0.05$).

Discussion

Sudden sensorineural hearing loss (SSHL) has been treated with systemic steroids traditionally, but a variety of methods are also applied in the treatment of SSHL [19]. In a recent report to evaluate the treatment of SSHL using a variety of treatment protocols, positive results were reported favoring systemic steroids, intratympanic steroids, batroxobin, magnesium, vitamin E, and HBO [19]. In another study, receiving HBO plus standard treatment (prednisone, dextran 40, diazepam, and pentoxifylline) and receiving standard treatment alone were compared. They found a greater rate of improvement, defined as gain of hearing of at least 10 dB on pure-tone average, in the HBO plus standard treatment [20]. On the other hand, cochlear effect of using HBO is still unclear and controversies still exist in the effectiveness of HBO in cochlear diseases. Because, so far, there are only a few studies to date have investigated the cellular changes that occur in inner ear after HBO [21]. Herein we investigated the auditory and histopathologic effects of HBO on the rat cochlea exposed to aminoglycoside with/without HBO. According to our results; in both control and HBO group, histopathological examination with H&E staining showed that any deterioration was not observed in cochlear hair cells ($p=0,710$). Additionally, with immunohistochemical examination, caspase-3 immunoreactivity was not observed. This finding indicates that HBO has probably no harmful effect on hair cells. On the other hand, it seemed that HBO has not any protective effect in a streptomycin-induced cochlear damage. Because, the histopathological results of the present study showed that almost similar degeneration was observed in streptomycin group and streptomycin plus HBO group ($p=0,864$). In addition to H&E staining, apoptotic cells in streptomycin group were observed in streptomycin plus HBO group with caspase-3.

Degeneration in cochlear hair cells and caspase-3 immunoreactivity in streptomycin plus HBO group was strongly associated with an increased apoptotic events in the cochlea. According to the accumulated informations obtained from previous reports, the most reasonable cause for the increase in apoptosis could be the excess free-radical agent accumulation. If free-radical agents are present at high concentrations, they are thought to act as a cytotoxin and can kill cells [4,5]. Then those dead cells (e.g., sensory hair cells) can be demonstrated histopathologically with caspase-3 as in the present study. In streptomycin and streptomycin plus HBO group, increased apoptotic cells were demonstrated in rat cochlea with caspase-3. This result suggested that HBO has not protective effect in a streptomycin-induced cochlear damage rat model. Our DPOAE results justified this finding. According to our results; the DPOAEs did not show any change (deterioration or improvement) in the control and HBO group. Hearing abilities of streptomycin-treated and streptomycin plus HBO rats were significantly deteriorated compared to other groups (control and HBO groups) within the tested range of frequencies. The analysis of the DPgram results revealed statistically significant differences between the streptomycin administration groups (streptomycin and streptomycin plus HBO) and others (control and HBO groups) ($p<0.05$). The results of streptomycin plus HBO group DPgrams are not significantly better than streptomycin group DPgrams. These results indicated that administration of HBO for streptomycin ototoxicity did not ameliorate hearing deterioration in rats.

The current study provides only limited insight regarding the effect of HBO against ototoxicity because of some reasons. Auditory brainstem response thresholds could not be obtained because of technical barriers; which is our major limitation. On the other hand, there was no possibility to assess the data with electron microscopy, which is another limitation. Therefore, further studies using those tools are needed in order to determine the exact effects of high pressure oxygen on cochlea.

In conclusion, high pressure oxygen use as a therapeutic method has been increasingly used in scientific studies. The present audiologic and histopathologic evidence indicates that HBO on cochlea has probably no harmful effect on hair cells. On the other hand, although HBO has antioxidant property, it seems to be not beneficial in a streptomycin-induced cochlear damage rat model.

Acknowledgments

The authors thank Yilmaz Palanci, Assistant Professor, from the Department of Public Health, Dicle University School of Medicine, for his valuable statistical assistance.

Competing interests

The authors declare that they have no competing interests.

References

1. Xie J, Talaska AE, Schacht J. New developments in aminoglycoside therapy and ototoxicity. Hear Res 2011;281(1-2):28-37.
2. Selimoglu E. Aminoglycoside-induced ototoxicity. Curr Pharm Des 2007;13(1):119-26.
3. Duggal P, Sarkar M. Audiologic monitoring of multi-drug resistant tuberculosis patients on aminoglycoside treatment with long term follow-up. BMC Ear Nose Throat Disord 2007;7:5.
4. Chen FQ, Schacht J, Sha SH. Aminoglycoside-induced histone deacetylation and hair cell death in the mouse cochlea. J Neurochem 2009;108(5):1226-36.
5. Wu WJ, Sha SH, McLaren JD, Kawamoto K, Raphael Y, Schacht J. Aminoglycoside ototoxicity in adult CBA, C57BL and BALB mice and the Sprague-Dawley rat. Hear Res 2001;158(1-2):165-78.
6. Oghan F, Apuhan T, Yılmaz F. Ototoxicity caused by topical administration of gentamicin versus tobramycin in rabbits. Int J Pediatr Otorhinolaryngol 2011;75(7):915-8.
7. Jung S, Wermker K, Poetschik H, Ziebura T, Kleinheinz J. The impact of hyperbaric oxygen therapy on serological values of vascular endothelial growthfactor (VEGF) and basic fibroblast growth factor (bFGF). Head Face Med 2010:22;6:29.
8. Yassuda CC, Righetti AE, Cury MC, Hyppolito MA, Oliveira JA, Féres O. The role of hyperbaric oxygen therapy (hot) as an otoprotection agent against cisplatin ototoxicity. Acta Cir Bras 2008;23(Suppl 1):72-6; discussion 76.
9. Vera-Cruz P, Ferreira M, Zagalo C, dos Santos JM, Aguas AP. Structure of the rat nasal mucosa after acute and chronic hyperbaric oxygen therapy. Undersea Hyperb Med 2010;37(2):125-32.
10. Camporesi RM: Hyperbaric oxygen therapy. A Committee Report. Undersea and hyperbaric medical society kensington MD USA; 1996.
11. Fujimura T, Suzuki H, Shiomori T, Udaka T, Mori T. Hyperbaric oxygen and steroid therapy for idiopathic sudden sensorineural hearing loss. Eur Arch Otorhinolaryngol 2007; 264(8):861-6.
12. Aslan I, Oysu C, Veyseller B, Baserer N. Does the addition of hyperbaric oxygen therapy to the conventional treatment modalities influence the outcome of sudden deafness? Otolaryngol Head Neck Surg 2002;126(2):121-6.
13. Nakashima T, Fukuta S, Yanagita N. Hyperbaric oxygen therapy for sudden deafness. Adv Otorhinolaryngol 1998;54:100-9.
14. Kuokkanen J, Virkkala J, Zhai S, Ylikoski J. Effect of hyperbaric oxygen treatment on permanent threshold shift in acoustic trauma among rats. Acta Otolaryngol 1997;529(suppl):80-2.
15. Ylikoski J, Mrena R, Makitie A, Kuokkanen J, Pirvola U, Savolainen S. Hyperbaric oxygen therapy seems to enhance recovery from acute acoustic trauma. Acta Oto-

laryngol 2008;128(10):1110-5.
16. Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. Circ Res 1999;84(3):253-6.
17. Institute of Laboratory Animal Research. Commission on Life Sciences. National Research Council. The Guide for the Care and Use of Laboratory Animals. 7. Ed. Washington DC: National Academy of Sciences; 1996.
18. Lin CD, Wei IH, Lai CH, Hsia TC, Kao MC, Tsai MH, Wu CH, Tsai MH. Hyperbaric oxygen upregulates cochlear constitutive nitric oxide synthase. BMC Neurosci 2011;12:21.
19. Conlin AE, Parnes LS. Treatment of sudden sensorineural hearing loss: I. A systematic review. Arch Otolaryngol Head Neck Surg 2007;133(6):573-81.
20. Topuz E, Yigit O, Cinar U, Seven H. Should hyperbaric oxygen be added to treatment in idiopathic sudden sensorineural hearing loss? Eur Arch Otorhinolaryngol 2004;261:393-6.
21. Xie J, Talaska AE, Schacht J. New developments in aminoglycoside therapy and ototoxicity. Hear Res 2011;281(1-2):28-37.